

AMENDMENTS TO THE CLAIMS

In the claims:

Claims 1-3 and 6 were the subject of the present Office Action. Please amend claims 1 and 3, cancel claims 2 and 6 without prejudice, and add new claims 27-36, as shown in the following listing of claims, which will replace all prior versions and listings of claims in the application. Claims 8-26 were withdrawn.

Listing of claims:

1. (currently amended) An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein the enzyme is selected from:

(A) ~~comprises the polypeptide having an amino acid sequence of SEQ ID NO: 1, or~~

(B) ~~a polypeptide having more than 50% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:~~

(1) ~~²⁵LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)~~

(2) ~~⁶⁷LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)~~

(3) ~~⁹²PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)~~

(4) ~~¹²⁸VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)~~

(5) ~~¹⁹⁹DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)~~

(6) ~~²⁵³RGGFXVLAVXHP₂₆₄ (SEQ ID NO: 28)~~

~~and comprising all of the conserved amino acid residues of SEQ ID NO: 1 that is:~~

~~L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108),~~

~~L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136),
P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166),
L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203),
A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225),
G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256),
V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO:
29).~~

2. (canceled)

3. (currently amended) The enzyme according to claim ~~[[1]]~~ 27, wherein the enzyme comprises the consensus amino acid sequence of ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27).

4. (canceled)

5. (canceled)

6. (canceled)

7. (canceled)

8. (withdrawn) A gene encoding amino acid sequence of nicotianamine synthase according to any one of claims 1-7.

9. (withdrawn) The gene according to claim 8 wherein said gene is cDNA.

10. (withdrawn) The gene according to claim 8 or 9 comprising having base sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14.
11. (withdrawn) The gene according to claim 8 or 9 comprising having base sequence shown in SEQ ID NO: 18, 20 or 22.
12. (withdrawn) A vector comprising containing gene according to any one of claims 8 – 11.
13. (withdrawn) The vector according to claim 12 wherein said vector is an expression vector.
14. (withdrawn) A transformant wherein said transformant is transformed by the vector according to claim 12 or 13.
15. (withdrawn) The transformant according to claim 14 wherein the foreign gene is a gene having base sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 22.
16. (withdrawn) The transformant according to claim 14 or 15 wherein the host is bacteria.
17. (withdrawn) The transformant according to claim 14 or 15 wherein the host is higher bacteria.
18. (withdrawn) A process for production of nicotianamine comprising using the transformant according to any one of claims 14 – 17.
19. (withdrawn) A plant wherein the gene according to any one of claims 8 – 10 is introduced.
20. (withdrawn) The plant according to claim 19 wherein said plant is seed.

21. (withdrawn) A fruit obtained by growing the plant according to claim 19 or 20.
22. (withdrawn) An antibody against nicotianamine synthase according to any one of claims 1 – 7.
23. (withdrawn) The antibody according to claim 22 wherein said antibody is polyclonal antibody.
24. (withdrawn) The antibody according to claim 22 wherein said antibody is monoclonal antibody.
25. (withdrawn) A method for extraction of nicotianamine synthase comprising extracting the said enzyme in the presence of thiol protease inhibitor at the extraction of nicotianamine synthase from the plant.
26. (withdrawn) The method according to claim 25 wherein the thiol protease inhibitor is E-64.
27. (new). An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein the enzyme:
- a. is a polypeptide having at least 50% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:
 - (1) ₂₅LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)
 - (2) ₆₇LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)
 - (3) ₉₂PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)
 - (4) ₁₂₈VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)
 - (5) ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)
 - (6) ₂₅₃RGGFXVLAVXHP₂₆₄ (SEQ ID NO: 28); and

b. has more than 25% of the nicotianamine synthase activity of an equivalent amount of the nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

28 (new). The enzyme of claim 27, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

29 (new). The enzyme of claim 27, wherein the polypeptide has more than 90% identity with an amino acid sequence of SEQ ID NO: 1.

30 (new). The enzyme of claim 27, wherein the polypeptide has more than 95% identity with an amino acid sequence of SEQ ID NO: 1.

31 (new). The enzyme of claim 27, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.

32 (new). The enzyme of claim 27, wherein the enzyme is isolated from a plant.

33 (new) The enzyme of claim 32, wherein the enzyme is isolated from barley.

34. (new) The enzyme of claim 32, wherein said enzyme is isolated from *Oryza sativa*.

35 (new). A mutated enzyme exhibiting nicotianamine synthase activity, wherein the enzyme:

a. is a polypeptide having more than 90% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:

(1) ₂₅LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)

(2) ₆₇LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)

(3) ₉₂PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)

(4) ₁₂₈VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)

(5) ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)

(6) ₂₅₃RGGFVXLAVXHP₂₆₄ (SEQ ID NO: 28); and

b. has more than 25% of the nicotianamine synthase activity of an equivalent amount of the nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

36 (new). The enzyme of claim 35, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203),

BEST AVAILABLE COPY

Docket No. 55107 (71526)

APPLICANT: S. Mori et al.

SERIAL NO: 09/674,337

FILED: July 26, 2001

Page 8 of 16

A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225),
G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256),
V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO:
29).
